

AD_____

Award Number: W81XWH-09-1-0746

TITLE: A Randomized Clinical Trial of Allopregnanolone for the Treatment of Severe Traumatic Brain Injury

PRINCIPAL INVESTIGATOR: Michael A. Rogawski, M.D., Ph.D.

CONTRACTING ORGANIZATION: University of California, Davis
Davis, California 95618-6134

REPORT DATE: October 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Service, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 01-10-2010	2. REPORT TYPE Annual	3. DATES COVERED (From - To) 30 SEP 2009 - 29 SEP 2010
---	--------------------------	---

4. TITLE AND SUBTITLE

A Randomized Clinical Trial of Allopregnanolone for the Treatment of Severe Traumatic Brain Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0746

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Rogawski, Michael A.; Natale, Joanne; Baynes, Kathleen; Diercks, Deborah; Muizelaar, Paul; Murphy, Randall; Pieribone, Vincent

rogawski@ucdavis.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California, Davis

Davis, California 95618-6134

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

There is strong experimental support for the concept that allopregnanolone will be safe and have beneficial effects on disability when administered as a treatment following acute traumatic brain injury (TBI). This study will provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with severe TBI. A phase II, fixed dose, placebo controlled, double blind, randomized clinical trial will be undertaken at the UC Davis Medical Center, a Level 1 trauma center. A subawardee (Medkura Pharmaceuticals assisted by SAFC) has been engaged to provide pyrogen-free allopregnanolone manufactured according to Current Good Manufacturing Practices (cGMP). Intravenous solutions of active drug and placebo will be formulated within the GMP Facility at the UC Davis School of Medicine. A subawardee, PRA International (a contract research organization), will be responsible for study management so that the trial will be compliant with FDA Good Clinical Practice requirements. Key research accomplishments during the reporting period include the development of a synthetic route for large scale manufacturing of pharmaceutical grade allopregnanolone of high purity. In addition, an HPLC assay method for allopregnanolone was developed.

15. SUBJECT TERMS

traumatic brain injury, clinical trial, allopregnanolone

16. SECURITY CLASSIFICATION OF:	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
---------------------------------	----------------------------	---------------------	---------------------------------

a. REPORT
U

b. ABSTRACT
U

c. THIS PAGE
U

UU

1 7

USAMRC

19b. TELEPHONE NUMBER (Include area code)

Table of Contents

	Page
I. Introduction.....	2
II. Body.....	3
III. Key Research Accomplishments.....	10
IV. Reportable Outcomes.....	10
V. Conclusion.....	10
VI. References.....	11
Appendices.....	13

Section I – Introduction

This study will provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with severe traumatic brain injury (TBI). A phase II, fixed dose, placebo controlled, double blind, randomized clinical trial will be undertaken at the UC Davis Medical Center (UCDMC), a Level 1 trauma center. A subawardee (Medkura Pharmaceuticals assisted by SAFC) has been engaged to provide sterile, pyrogen-free allopregnanolone manufactured according to Current Good Manufacturing Practices (cGMP). Intravenous solutions of active drug and placebo will be formulated within the GMP Facility at the UC Davis School of Medicine. A subawardee, PRA International (a contract research organization), will be responsible for study management so that the trial will be compliant with FDA Good Clinical Practice requirements. A study manager will provide oversight of PRA and the conduct of the trial. As part of their responsibilities, PRA International will provide safety management and reporting to the FDA. This will ensure strict compliance with IND requirements. The study involves the use of human subjects; the estimated enrollment is 136 subjects, with equal allocation to allopregnanolone and placebo.

This research seeks to demonstrate that administration of allopregnanolone following severe TBI is safe and reduces disability compared to placebo. The primary outcome measure of the trial is the Glasgow Outcome Scale–Extended (GOS-E). The study is designed to detect a 1 point improvement. A 1 point improvement is clinically significant as such an improvement would place the GOS-E at the level reported for patients with mild TBI (GCS 13–15) (Hudak et al., 2005). Several authors have found that greater disability and handicap as measured by the GOS and GOS-E are associated with subjective self-reports of poorer outcome. Specifically, individuals with poorer outcome as measured by the GOS or GOS-E had a higher frequency of depressive symptomatology (McCleary et al., 1998; Wilson et al., 2000). Poor outcome is also associated with reduced mental well-being and problems in neurobehavioral functioning (Wilson et al., 2000). Overall, the improved outcome contemplated by allopregnanolone treatment is expected not only to be associated with improved neurological function but also with an improved subjective sense of satisfaction with life (Wilson et al., 2000). Since it is difficult to conduct clinical research in a war zone, we have chosen to conduct this research in a civilian setting. Nevertheless, we believe that the results obtained will be applicable to the use of allopregnanolone in a military situation. Such application has the potential to have a dramatically positive impact on the function, wellness, and overall quality of life for military Service members affected by severe TBI. Caregivers and families will also be positively impacted since affected Service members with less disability will require less demanding care. The Brain Injury Association of America estimates that the long-term cost of care for a person with severe TBI is \$4.1 to 9 million. Such an individual may require 5 to 10 years of rehabilitation and follow-up services. Therefore, in addition to providing improved function, well-being and overall quality of life, the improvement contemplated by allopregnanolone treatment should result in substantial societal cost savings.

There is strong experimental support for the concept that allopregnanolone will have beneficial effects in TBI. Allopregnanolone, a neurosteroid that acts as a powerful modulator of GABA_A receptors, has anticonvulsant and anesthetic activity but is free of the hormonal actions of progesterone (Rogawski and Reddy, 2004), another agent currently being studied in the

treatment of TBI. Recent studies have demonstrated that allopregnanolone is efficacious in enhancing neurobehavioral recovery and decreasing TBI-induced neuronal death (Djebali et al., 2004; 2005; He et al., 2004ab; Ciriza et al., 2006; Sayeed et al., 2006). These studies support the clinical trial to be conducted in this project.

Section II – Body

During the current reporting period, tasks were completed in regard to negotiation of subawards to contractors (PRA International and Medkura Pharmaceuticals), development of a clinical study protocol, study drug manufacturing (Medkura Pharmaceuticals with the assistance of SAFC), development of an intravenous formulation, development of bioanalytical methods, and the preparation of documentation for submission to regulatory authorities [(U.S. Food and Drug Administration (FDA) and institutional review board (IRB)].

Kick-off Meeting. Immediately following the notification of award on September 30, 2009 project teams were constituted to carry out the various tasks required to prepare for and conduct the clinical study. On October 8, 2009 the entire project team assembled for a kick-off meeting at UC Davis Medical Center. The kick-off meeting continued on October 9. The participants included all of the key study personnel and representatives of the subcontractors. Dr. Michael Rogawski (principle investigator) provided an initial introduction. Dr. Vincent Pieribone (consultant) described the structure of the project teams and the task to be completed during the kick-off meeting by working groups that would be constituted. Dr. Joanne Natale (co-investigator and medical monitor) described the clinical trial. The remainder of the day was spent with each of the working groups planning each of their task. The working groups included: (1) Clinical Protocol Development Working Group, (2) Budget Working Group, (3) Regulatory Working Group, and (4) API Manufacturing/Formulation and Drug Supply Working Group. On the second day of the kick-off meeting subgroup discussed the database/statistical plan and informed consent.

Negotiation of Subawards. Our initial task during the early months of the project was the negotiation of subawards with contractors. This process began on October 7, 2009, with the engagement of Sue Blair, Contracts & Grants Officer, UC Davis Office of Research (Sponsored Programs). Ms. Blair met with representative of our subcontractors PRA International and Medkura Pharmaceuticals at the Kick-off Meeting on October 8. She worked closely with PRA International to develop an acceptable subcontract. Agreement was reached on November 9, 2009 except for one critical contract requirement. PRA legal mandated an indemnification provision whereby PRA was indemnified for any liabilities that might occur during the trial, such as subject injuries. Inquiries were made to the Department of Defense and it was determined that the federal government does not provide such indemnification. Similarly, university officials reported that the university can only indemnify for the negligence of its own officers, agents and employees (per the university's Standing Orders), and it was necessary to determine whether UC Davis Health System would take on the additional indemnification of PRA. Meetings were subsequently held with Health System counsel and negotiations proceeded between Health System counsel and PRA's director of contracts and legal affairs.

A solution to the “indemnification gap” was provided by the development of a letter of mutual indemnity between Medkura and PRA, which was completed on February 25, 2010.

Negotiations proceeded between UC Davis Health System and PRA resulting in a final PRA agreement, which was signed on March 4, 2010.

On December 28, 2009, Ms. Blair provided Medkura with a detailed subcontract and scope of work. Negotiations proceeded with Medkura and were concluded on January 24, 2010 with the presentation of a mutually agreeable Medkura subcontract, which was signed on February 19, 2010. At this point agreements with both subcontrators were in place. Particularly critical at this stage was the Medkura subcontract, which allowed many aspects of trial development to proceed, including the development of documents for filing with regulators and the manufacturing of sufficient quantities of allopregnanalone, the active pharmaceutical ingredient (API), under cGMP (current good manufacturing practices).

Clinical Study Protocol. In parallel with the subcontract negotiations, the protocol working group completed and finalized the clinical study protocol. The protocol working group was led by Joanne Natale, M.D., Ph.D. Other members of the subcommittee include Michael A. Rogawski, M.D., Ph.D., Julia Tsai, Ph.D., Vincent Pieribone, Ph.D., Nancy Rudisil, R.N., Deborah Diercks, M.D., Daniel Nishijima, M.D., and Gerhard Bauer, B.S.

The protocol was extensively reviewed by four outside reviewers: Kia Shahlaie, M.D., Ph.D. (University of California, San Francisco), Anne-Marie Guerguerian, M.D. (University of Toronto), Claudia Robertson, M.D. (Baylor College of Medicine), and Paul Vespa, M.D., (University of California, Los Angeles). All reviewer comments were evaluated by the subcommittee and alterations were made when the committee deemed necessary. The clinical trial protocol was used in the development of the Pre-IND Package described below and the UC Davis IRB filing.

Pre-IND Meeting Information Package and IND. Medkura Pharmaceutical and Camargo Pharmaceutical Services, Inc., a contract research organization engaged by Medkura with expertise in 505(b)(2) filings, reviewed the protocol along with an extensive dossier of information on allopregnanolone that was provided by the UC Davis project team. During months of discussion with Camargo a Pre-IND meeting information document was developed which will serve as the basis for the IND filing that will be made after the Pre-IND meeting. The Pre-IND Package includes a request for a Type B (Pre-IND) face-to-face meeting with the FDA in accordance with 21 CFR Part 312.82 to discuss the development plans of intravenous allopregnanolone for the treatment of patients with acute TBI. It provides a statement of the purpose and objectives of the meeting and a preliminary proposed agenda. A set of regulatory, clinical pharmacology, and clinical questions are provided. In addition, the Pre-IND Package includes an extensive dossier of supporting information and documentation for each of the questions to the FDA. The dossier also includes a description of the scientific rationale for the study and an account of the regulatory history mainly focusing on progesterone. The remainder of the Pre-IND Package is a detailed summary of the available scientific literature supporting the safety and potential utility of allopregnanolone in the treatment of TBI.

Filing of the request for Pre-IND meeting is scheduled for the week of November 1, 2010. We expect a face-to-face meetings with the FDA, which will be attended by representatives of the UC Davis study team and also by Camargo.

Development of Manufacturing Method. A major challenge has been the manufacturing of the study drug allopregnanolone (API), which had not previously been manufactured in the quantities and to the purity specifications required for this clinical trial. Moreover, the requirement that the manufacturing be conducted according to cGMP markedly increased the difficulty. Our subcontractor Medkura was tasked with the responsibility of providing the API to our specifications. Medkura developed the synthetic and bioanalytical methods as contracted but because a key employee became seriously ill they were unable to undertake the actual manufacturing. It became necessary for Medkura to engage a subcontractor (SAFC, a subsidiary of Sigma-Aldrich, Madison, WI) to assist with the development of the synthesis and to be responsible for cGMP production. On June 17, 2010, manufacturing by SAFC was initiated with a kick-off meeting by teleconference. Members of the UC Davis project team and personnel from Medkura and SAFC had weekly (Friday morning) teleconferences to monitor SAFC's progress and so that Medkura could provide technical assistance on an ongoing basis. The UC Davis project team, Medkura and SAFC communicated frequently at other times apart from the weekly calls during the process development and manufacturing.

The synthetic pathway as originally conceived by Medkura is as show in Fig. 1 below.

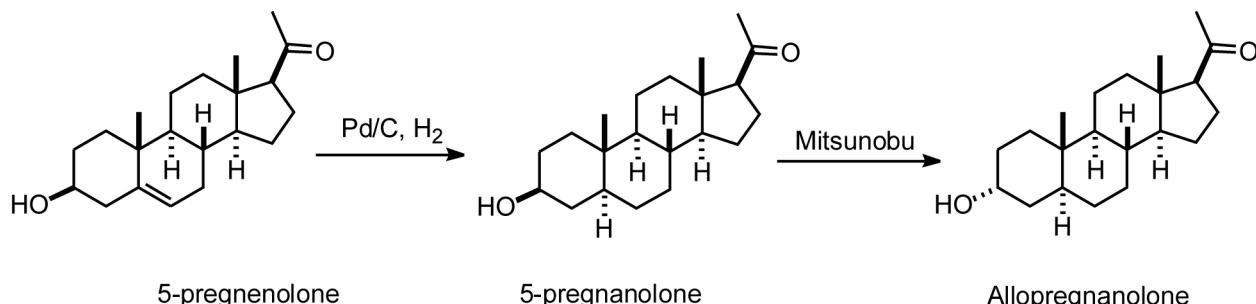


Fig. 1. Synthetic scheme for preparation of allopregnanolone (3 α -hydroxy-5 α -pregnan-20-one).

Medkura had found a difficulty with the contamination of the final product with the byproduct triphenylphosphine oxide. This was present at a concentration of several percent and could not be removed by repeated crystallization. Further work on the development of the manufacturing process was continued by SAFC. Specific details for each of the reaction steps are as follows:

Step 1. 5-Pregnenolone to 5-Pregnanolone

Medkura originally demonstrated that this step could be accomplished using a Paar apparatus at 30-40 psi of hydrogen using a dry 10% palladium on carbon catalyst. Medkura under these conditions found that the yield was almost quantitative and that less than 1% of byproducts were formed. Medkura also found that at lower pressures hydrogenation was much slower. SAFC used a wet rather than a dry catalyst for safety reasons. They ran both laboratory scale and a larger qualification runs, and discovered several problems. The solution temperature would upon

adding hydrogen rise to 50-60 °C and this would result in the formation of substantial amounts of impurities. Lower hydrogen pressures and shorter reaction times did not solve this problem. Eventually, SAFC determined that by careful cooling these issues could be reduced and that pure material could be produced in good yield. However, there seemed to be substantial batch-to-batch variability in the hydrogenation conditions, probably due to slight changes in catalyst activity. For example, an initial 200 gm qualification run proceeded slowly. Accordingly, it was tried again heating from only 20 to 25 °C. This produced a temperature spike to about 50 °C which resulted in product which contained about 15% of various unidentified diastereomeric impurities. Accordingly great care was taken in the final cGMP run to cool the reaction and avoid temperature spiking.

There were also considerable experimentation required to identify the appropriate solvent for the reaction. Due to the relatively modest solubility of the starting material in ethanol, it was considered desirable to investigate the use of other solvents and cosolvents. These included (a) 1:1:0.2 ethanol/tetrahydrofuran/acetic acid, (b) 1:1 ethanol/2-methyl tetrahydrofuran, (c) 20% (v/v) dichloromethane in ethanol, (d) 20% methanol in dichloromethane. Of these system (d) gave complete conversion and was selected for the final GMP run. Filtration of the catalyst through celite with the use of glass fiber filter paper was found to be sufficient to reduce levels of palladium and other metals in the final product to an acceptable (ppm) range. Some issues were found with regard to lowered yields due to retention of some material on the celite cake, but this could be remedied by washing with excess hot solvent.

Step 2. Mitsunobu Reaction

Medkura had previously found that using triphenylphosphine the Mitsunobu reaction proceeded well but residual triphenylphosphine oxide was formed which was present at a level of about 5% in the final product. This was found to be impossible to remove by repeated recrystallization. Thus, the use of triphenylphosphine in the Mitsunobu reaction was a potential concern. Initially some effort was made to substitute tributylphosphine for triphenylphosphine oxide. However, this effort was not successful.

As other phosphines are substantially more expensive it was not feasible to adopt an alternative phosphine. It was therefore necessary to develop a means of removing the triphenylphosphine oxide by some appropriate process. A suggestion by Dr. Robert Purdy (San Diego Veterans Administration Hospital) was to make the 2,4-dinitrobenzoyl ester of pregnanolone, which was more readily separated in his experience from the triphenylphosphine oxide. This concept was modified to use *p*-nitrobenzoic acid in the Mitsunobu reaction instead of trifluoroacetic acid as the donor molecule. This procedure resulted in excellent yields of the *p*-nitrobenzoyl ester, which could be freed almost completely from the triphenylphosphine oxide by a single recrystallization.

The revised synthetic scheme is shown in Fig. 2.

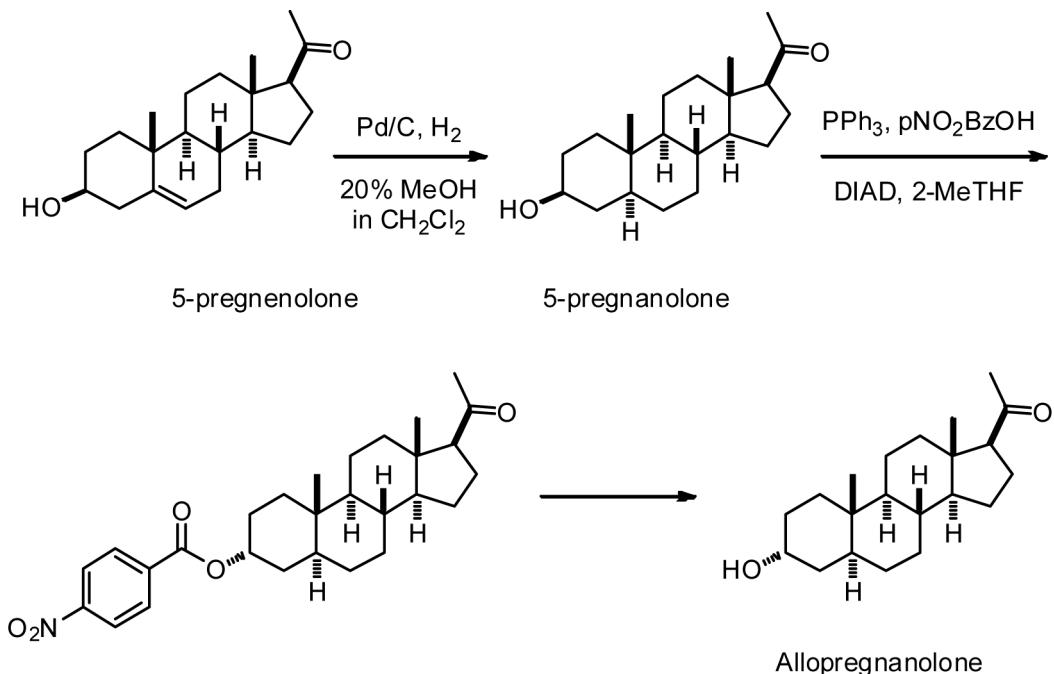


Fig. 2. Revised synthetic scheme with *p*-nitrobenzoic acid (*p*NO₂BzOH) in the Mitsunobu reaction.

Step 3. Hydrolysis

Multiple attempts using different reaction conditions were tried to hydrolyze the *p*-nitrobenzoic acid derivative to the final product allopregnanolone. Reaction 388-130 utilized 1 N NaOH in methanol and tetrahydrofuran at a temperature of 35 °C. This resulted in a 40% isolated yield for the main lot with the product displaying 93% HPLC purity. The main impurity was *p*-nitrobenzoic acid. The low yield was due to the fact that ethyl acetate was used as the crystallization and wash solvent. The product was found to be more soluble in ethyl acetate than originally envisioned and this resulted in the low yield.

Reaction 388-131 was run on 15 g scale and was complete after about 5 hours. The work-up of the reaction was split and half of the organic layer was retained. A modified work-up compared to reaction 388-130 was used on the other half in which the organic layer was washed with twice as much NaOH as in 388-130, and then washed twice with water. The organic layer was concentrated to 10 volumes, and 10 volumes of heptane were charged over 10 minutes while the slurry was stirring. The slurry was then concentrated to 10 volumes, stirred at room temperature overnight, and then cooled to 0 °C for 4 hours. After filtration and heptane washing, the product was dried at 40 °C overnight. Residual solvent analysis of the slurry supernatant prior to filtration showed that it contained 28% ethyl acetate. Based on these experiments, the optimal conditions were determined to be as follow: material was redissolved in ethyl acetate/methanol, washed twice with 1 N NaOH, twice with water, and concentration until ~7 volumes of ethyl acetate remained. The slurry was then heated to fully dissolve the material and heptane was added to precipitate allopregnanolone. After isolation and drying, a 59.2% recovery was obtained.

The purity of the isolated material is indicated by the results of a typical HPLC run illustrated in Figs. 3 and 4. The impurities noted will be identified using chemical methods and also by running known compounds on HPLC.

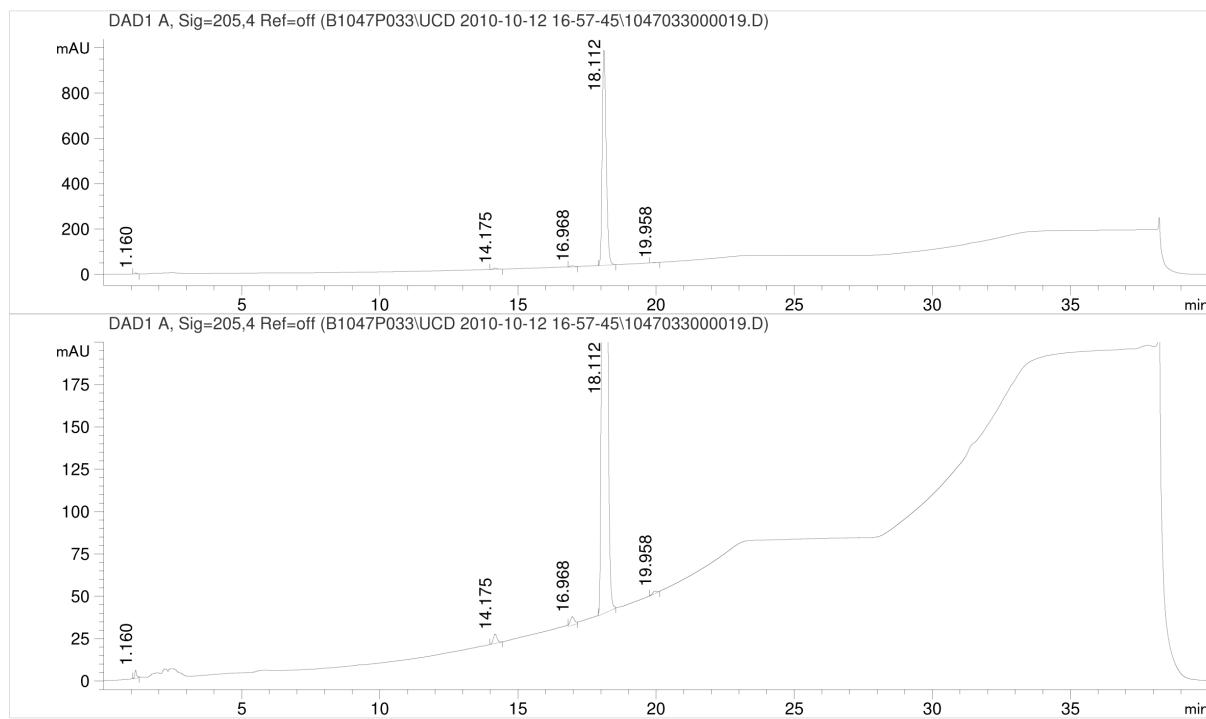


Fig. 3. Representative HPLC analysis of qualification run material late in the development of the synthetic route. Allopregnanolone is at retention time 18.112. Four impurities are noted.

#	Meas.	R	Height	Width	Area	Area %
1	1.160		4.822	0.068	22.426	0.232
2	14.175		5.558	0.147	52.750	0.545
3	16.968		4.963	0.190	56.599	0.585
4	18.112		949.925	0.167	9525.206	98.461
5	19.958		1.563	0.183	17.153	0.177

Fig. 4. Quantification of the components from the HPLC trace shown in Fig. 3. Four unidentified impurities were present, representing 1.539%. These components were substantially reduced in the final production run to less than 0.3% each. The levels meet FDA standards as less than 1 mg is expected to be administered daily.

Step 4. Final Recrystallization

Unfortunately, in later runs small amounts of impurities, presumably formed in the Mitsunobu reaction, begin to appear in the final product. Therefore renewed attempts were made at

recrystallization to remove these impurities. It was determined that a recrystallization of allopregnanolone from 10 L/kg of ethanol and 7 L/kg of water was able to increase the product purity to well within the specification of 98% purity. Manufacturing process development was therefore deemed successful.

cGMP Manufacturing. Upon completion of the major steps in the development of the manufacturing process, it was possible to begin cGMP manufacturing on October 1, 2010. This was performed using 19.8 kg of 5-pregnenolone as starting material. The 5-pregnenolone was dissolved in 20% methanol/dichloromethane, charged to 0.99 kg of 10% palladium on carbon, and allowed to hydrogenate under 20 PSI at 20 ± 5 °C. At 30 hours the reaction was sampled and deemed complete with only 0.2% 5-pregnenolone remaining relative to 5-pregnanolone. The 10% palladium on carbon was filtered off as the product containing solution was transferred into a 200-gallon reactor. A solvent switch to 2-methyltetrahydrofuran (2-Me-THF) was performed. Subsequently, 13.5 kg of 4-nitrobenzoic acid and 20.4 kg of triphenylphosphine were charged to the reactor. While operators were cooling the slurry to 0 ± 5 °C, solids settled to the bottom due to inadequate stirring. To aid in better agitation, 43.0 kg of 2-methyltetrahydrofuran was added to the reactor. Once sufficient agitation was reached and the temperature was within range, 18.2 kg of DIAD was slowly added to the reactor. The reaction mixture was warmed to 20 ± 5 °C and the Mitsunobu reaction began.

The Mitsunobu reaction stirred at 20 ± 5 °C for 15 hours and 50 minutes, until 0.1% 5-pregnanolone remained relative to 4-NO₂Bz-allopregnanolone. A solvent switch was performed from 2-Me-THF to IPA until residual 2-Me-THF remaining was < 0.1%. The slurry was filtered, and the filter cake was washed twice with isopropyl alcohol (IPA). 2-propanol was used for the remainder of the process.

The 4-NO₂Bz-allopregnanolone was isolated and dried for 66 hours and 28 minutes, and was deemed 1% dry by thermogravimetric analysis. The product was reslurried for 6 hours and 15 minutes, washed with IPA, isolated, and allowed to dry for 18 hours and 8 minutes. It was deemed 1% dry by TGA. A sample was analyzed by HPLC and the purity was 100% (rounded from 99.7%) with <0.01% triphenylphosphine oxide. The product was discharged with a net weight of 24.9 kg representing an 85% yield going into step 3.

The product was slurried in THF and MeOH with 1M NaOH added over 75 minutes, maintaining internal temperature below room temperature. The hydrolysis reaction was allowed to stir for 8 hours and 47 minutes until 0.2% (uncorrected) 4-NO₂Bz-allopregnanolone remained relative to allopregnanolone. The aqueous workup was performed very slowly due to large emulsions that were observed. The organics were polish filtered once a pH of 7 of the water washes was reached, and the distillations were performed until 0.6% residual THF remained. The recrystallization was performed and allopregnanolone was dried. The final product is 99.26% pure which fully met our purity specification (Appendix 1). A certificate of analysis of the preliminary (qualification run) material is presented in Appendix 2. The certificate of analysis of the significantly higher purity production run material is expected to be available in approximately 2 weeks.

Analytical Method Development. Medkura developed an HPLC separation method for allopregnanolone using an Agilent LiChrospher 100 reverse phase (C18) 250 × 4 mm, 5 μ

column. The mobile phase was acetonitrile:methanol (325:25). The separation is run at room temperature and detection utilized UV at 205 nm. Total run time is 30 min and equilibration time is 4 min.

Formulation. An intense effort has been focused on the development of the final clinical formulation of the drug product. This has involved several members of the development team including Drs. Rogawski, Pieribone, Tsai, and Murphy, and Mr. Bauer. We have compared various formulation strategies, including the formulation of 5 mg/ml allopregnanolone in a 25% human albumin solution as originally contemplated. As we have investigated the use of human albumin, we have become concerned that it may not be the safest solubilizing agent and carrier. Albumin is derived from human plasma and as such may contain infectious agents, such as viruses, that can cause disease. Other issues include the risk of developing circulatory overload and pulmonary edema with rapid administration. Adverse reactions such as nausea, fever, chills or urticaria can occur although they are rare. Apart from the safety issues, the albumin formulation is less than ideal for deployment into the field, such as in a combat situation. Therefore, we have investigated an alternative formulation using a cyclodextrin, such as hydroxypropyl- β -cyclodextrin (HPBCD), which has a good safety record and is likely to be considered safe by the FDA for intravenous administration at high doses. We are developing a dry complex between the API and HPBCD that can be reconstituted for intravenous infusion. If successful, we plan to utilize this material which will undergo final formulation at the UC Davis GMP facility immediately prior to administration.

Section III – Key Research Accomplishments

- Developed a synthetic route for large scale manufacturing of pharmaceutical grade allopregnanolone.
- Developed HPLC assay methods for allopregnanolone.

Section IV – Reportable Outcome

None.

Section V – Conclusion

This project seeks to provide initial data on the safety and effectiveness of allopregnanolone in improving neurobehavioral outcome and reducing mortality in adults with severe TBI through a phase II, fixed dose, placebo controlled, double blind, randomized clinical trial. During the first year of this project we have developed a clinical trial protocol. Using the protocol and an extensive base of information on allopregnanolone, an IND package was developed for submission to the FDA. A document was also developed for submission to the local IRB and the United States Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protection Office. We have therefore completed most of the task defined in the Statement of Work, except that the IRB application has not yet been approved. Also, enrollment and data collection has not yet begun. Assuming that regulatory review is uneventful, this could begin as early as month 16 instead of month 10 as indicated in the Statement of Work. The most

significant risk to meeting this timeline is the possibility that the FDA issues a clinical hold, which would require us to provide additional information to assure the agency of the safety of the trial.

A major challenge was manufacturing of the study drug. The original contractor (Medkura) was unable to complete this task and an intense effort was mounted to identify an alternative manufacturer. After a global search, only one organization was identified with the capability and interest in pursing the project. This organization, SAFC (Sigma-Aldrich), was subcontracted by Medkura. With the assistance of Medkura and the intense involvement of key members of the UC Davis project team and consultants, extensive experimentation led to the successful identification of a synthetic route for manufacturing pharmaceutical grade allopregnanolone. With this critical hurdle overcome, the regulatory documents can be filed in anticipation of commencing patient enrollment.

The research conducted in year 1 of this project has advanced the development of a potential treatment approach for adults with severe TBI. The methods we have discovered for the manufacture of pharmaceutical grade allopregnanolone are applicable to others who seek to manufacture allopregnanolone for clinical trials in TBI. The methods are also applicable to the eventual production of allopregnanolone for deployment as a treatment agent if approved for use by regulatory authorities.

Section VI – References

Ciriza I, Carrero P, Frye CA, Garcia-Segura LM (2006) Reduced metabolites mediate neuroprotective effects of progesterone in the adult rat hippocampus. The synthetic progestin medroxyprogesterone acetate (Provera) is not neuroprotective. *J Neurobiol* **66**:916-928.

Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG (2005) The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma* **22**:106-118.

Djebaili M, Hoffman SW, Stein DG (2004) Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. *Neuroscience* **123**:349-359.

He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG (2004a) Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol* **189**:404-412.

He J, Hoffman SW, Stein DG (2004b) Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. *Restor Neurol Neurosci*. **22**:19-31.

Gould S, Scott RC (2005) 2-Hydroxypropyl- β -cyclodextrin (HP- β -CD): a toxicology review. *Food Chem Toxicol* **43**:1451-1459.

Hudak AM, Caesar RR, Frol AB, Krueger K, Harper CR, Temkin NR, Dikmen SS, Carlile M, Madden C, Diaz-Arrastia R (2005) Functional outcome scales in traumatic brain injury: a comparison of the Glasgow Outcome Scale (Extended) and the Functional Status Examination. *J Neurotrauma* **22**:1319-1326.

McCleary C, Satz P, Forney D, Light R, Zaucha K, Asarnow R, Namerow N (1998) Depression after traumatic brain injury as a function of Glasgow Outcome Score. *J Clin Exp Neuropsychol* **20**:270-279.

Rogawski MA, Reddy DS (2004) Neurosteroids: Endogenous modulators of seizure susceptibility. In, *Epilepsy: Scientific Foundations of Clinical Practice* (Rho JM, Sankar R, Cavazos J, eds.), Marcel Dekker, New York, pp. 319-355.

Sayeed I, Guo Q, Hoffman SW, Stein DG (2006) Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med* **47**:381-389.

Wilson JTL, Pettigrew LEL, Teasdale GM (2000) Emotional and cognitive consequences of head injury in relation to the Glasgow outcome scale. *J Neurol Neurosurg Psychiatry* **69**:204-209.

Appendix 1 – Active Pharmaceutical Ingredient Specification

SAFC PHARMA
Madison, WI 53711

SPECIFICATION
Dept. 200I

Page: 1

Allopregnanolone (UCD)

PRODUCT ID: UCD

FORMULA: C₂₁H₃₄O₂

MW: 318.49

CAS NUMBER: NA

CATEGORY: 4

SAMPLING: QC TEST: 2.0g

STORAGE: Store at controlled room temperature

2.0g for microbial limits
200mg for bacterial endotoxin
– Use sterile containers for
microbial limits and bacterial
endotoxin sampling

RESERVE: 8.4g

RETEST: 1 year from date of manufacture

PACKAGING

Product: Primary: Polyethylene charge bag with gasket, end cap, and clamp, size appropriate
Secondary: 4 mil Polyethylene bag, size appropriate
Tertiary: 30 Gallon open top plastic drum, RMS-006129

QC Sample: Primary: Type III amber glass jar with Teflon lined screw cap, size appropriate
Secondary: 4 mil Polyethylene bag, size appropriate

Reserves: Same as product packaging configuration

SPECIFICATIONS

APPEARANCE:	White to off-white powder
IDENTIFICATION (FT-IR):	Conforms to standard
¹H NMR:	Conforms to standard
IDENTIFICATION (HPLC):	Retention time ratio within 0.98 to 1.02 of standard
ASSAY (HPLC):	NLT 98.0%
IMPURITIES (HPLC):	No impurities detected greater than or equal to 1.0%
RESIDUAL SOLVENTS:	Methanol: NMT 3000ppm Dichloromethane: NMT 600ppm 2-Methyl-tetrahydrofuran: NMT 5000ppm Isopropyl alcohol: NMT 5000ppm Tetrahydrofuran: NMT 720ppm Heptane: NMT 5000ppm Ethyl acetate: NMT 5000ppm
WATER CONTENT:	Report
RESIDUE ON IGNITION:	NMT 0.1%

DSC:	Report onset
HEAVY METALS (ICP-MS):	Palladium: NMT 50 ppm NMT 10 ppm total USP metals (mercury, lead, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum)
MICROBIAL LIMITS:	Total aerobic microbial count: NMT 100 CFU/g
BACTERIAL ENDOTOXIN:	NMT 1.0 EU/mg

TESTING

APPEARANCE:	200-100
IDENTIFICATION (FT-IR):	200-227 Use KBr HATR method.
¹H NMR:	200-221 Use CDCl ₃ as the solvent.
IDENTIFICATION, ASSAY, and IMPURITIES (HPLC):	OP-018724
RESIDUAL SOLVENTS:	200-992
WATER CONTENT:	200-232 Analyze a sample of approximately 100-150mg dissolved in 4.0ml methanol using the solution technique. Perform in triplicate with AG reagent.
RESIDUE ON IGNITION:	200-248; <USP 281> Use approximately 1.0g sample.
DSC:	100-232 Method conditions: Ramp from 25-350°C at 10°C/min with N ₂ at 50mL/min.
HEAVY METALS (ICP-MS):	Send approximately 250mg sample for analysis of palladium and USP metals (mercury, lead, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum) to Exova at 9240 Santa Fe Springs Road, Santa Fe Springs, CA, 90670. Reference Exova Job No. 125857 on the test request form.
MICROBIAL LIMITS:	USP <61> Send approximately 2.0g to SGS Life Science Services at 616 Heathrow Drive, Lincolnshire, IL, 60069. Request "Total aerobic microbial count" to be performed per USP <61>. Include the Assignment No. 0981010325 on the test request form.
BACTERIAL ENDOTOXIN:	USP <85> Send approximately 200mg to Associates of Cape Cod at 124 Bernard E. St. Jean Drive, East Falmouth, MA, 02536. Request testing to be performed per IC Number 1010-063.

SHIPPING INSTRUCTIONS	Per OP 500-401 For IATA, classify as: Toxic solid, organic, n.o.s. Allopregnanolone UN 2811, Packaging Group II
------------------------------	---

Appendix 2 – Certificate of Analysis of Analysis of Qualification Run Material



| Inspiring Science

645 Science Dr., Madison, WI 53711
Tel (608) 233-3115 Fax (608) 233-6873

CERTIFICATE OF ANALYSIS

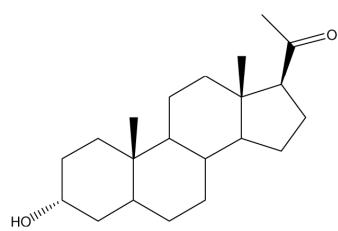
Material: Allopregnanolone (UCD)

Empirical Formula: C₂₁H₃₄O₂

Lot No.: 1010398010

Molecular Weight: 318.49

Structure:



Appearance:

White powder

HPLC Purity:

98.56%

Impurities: RRT 0.06 = 0.22%
RRT 0.78 = 0.55%
RRT 0.94 = 0.52%
RRT 1.10 = 0.17%

DSC Melt:

Onset: 174.9°C

¹H NMR:

Consistent with structure

FT-IR:

Consistent with structure

Water:

< 0.15%

Residual Solvents:

Methanol: Not detected
Isopropyl Alcohol: Not detected
Dichloromethane: Not detected
Tetrahydrofuran: 13ppm
Ethyl Acetate: 47ppm
2-Methyl THF: Not detected
Heptane: 131ppm

Not detected (< 0.1ppm)

Pd, ICP-MS:

Cu: 0.26ppm

Mo: 0.08ppm

Ag: 0.06ppm

All other elements (Sb, As, Bi, Cd, Pb, Hg, Sn) Not detected (< 0.1ppm each)
< 0.1%

Residue on Ignition:

TBD

Endotoxin:

TBD

Microbial Limits:

TBD

Storage: Store at ambient room temperature.

Handling: Exercise caution in the handling and formulation of this product.

Manufacture Date: October 13, 2010

Note: This material is for Research and Development use only and is not intended for use in humans. SAFC assumes no liability for damage resulting from handling, contact or misuse of this product.

10/20/10
TMJ/CMH